PATENT COOPERATION TREATY

23.OCT.2001* 1936

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P38044WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/02652	10/07/2000	23/07/1999
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant UNIVERSITY OF SHEFFIELD et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/02/2001	15.10.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Rojo Romeo, E Telephone No. +49 89 2399 7321

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/02652

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-18 as originally filed

Claims, No.:

1-20 with telefax of 28/09/2001

Drawings, sheets:

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-20
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-16, 18-20
	No:	Claims	17 (see separate sheet)

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of this report

The Applicant's arguments filed with his letters of September 6th, 10th and 28th 2001 were carefully taken into consideration for the establishment of the present report.

The replacement of CD154 ligand by CD154 was a correction of an obvious mistake (as stated at page 1 of the specification as filed: "The invention herein described relates to cells and/or tissues and/or organs which do not naturally express the cell surface receptor CD40 ligand, CD154".

The present set of claims complies with Art 19(2) EPC.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Novelty (Art. 33(2) PCT)
   None of the documents cited in the International Search Report discloses the claimed subject-matter. The current set of claims is thus considered novel over these documents.

2. Inventive step (Art. 33(3) PCT)
   Reference is made to the following documents cited in the International Search Report:
   D1: GAINER A. L. ET AL.: 'IMPROVED SURVIVAL OF BIOLISTICALLY TRANSFECTED MOUSE ISLET ALLOGRAFTS EXPRESSING CTLA4-IG OR SOLUBLE FAS LIGAND' TRANSPLANTATION, vol. 66, no. 2, 27 July 1998 (1998-07-27), pages 194-199, XP000877391 ISSN: 0041-1337
   D2: GRUSS H.-J. AND DOWER S. K.: 'Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas' BLOOD, vol. 85, no. 12, 15 June 1995 (1995-06-15), pages 3378-3404, XP002094502 ISSN: 0006-4971

   D1 can be considered as the closest prior art since it discloses the expression of members of the TNF family in transfected mouse islet cells leading to a protective effect on allograft survival. The problem underlying the present application is the provision of alternative molecules for expression in cells to be transplanted for

protection of said transplanted cells. The solution provided by the present application is the expression of CD154.
D2 reviews the TNF ligand superfamily: they are all essential for T-cell costimulation and activation. However, there was no hint from prior art that the expression of CD154 at the cell surface of a cell which does not express it naturally would lead to immune suppression.

None of the documents cited in the International Search Report would thus have allowed the skilled person to achieve the subject-matter of the present application in an obvious manner. Therefore, inventive step can be acknowledged for the present set of claims.

3. Industrial applicability (Art. 33(4) PCT)
For the assessment of the present claim 17 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VI

Certain documents cited

D1: WO 00 12138 A (DIACRIN INC) 9 March 2000 (2000-03-09)
international publication date: 09.03.00
international filing date: 31.08.99
priority data: 31.08.98

Re Item VII

Certain defects in the international application

Claims 18-20 refer to the cell of claim 1. Claim 1 is however a use claim, not a product claim.

CORRECTED VERSION*

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, 15/62, C07K 14/48, 14/52, 14/525, 14/705, C12N 15/86, A61K 48/00, 38/17, 35/12

A3

(11) International Publication Number: WO 98/26061

(43) International Publication Date: 18 June 1998 (18.06.98)

(21) International Application Number: PCT/US97/22740

(22) International Filing Date: 8 December 1997 (08.12.97)

(30) Priority Data:

60/032,145	9 December 1996 (09.12.96)	US
08/982,272	1 December 1997 (01.12.97)	US

(81) Designated States: AT, AU, BR, CA, CH, CN, DE, DK, ES, FI, GB, IL, JP, KR, LU, MX, NO, NZ, PT, RU, SE, SG, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(88) Date of publication of the international search report:
19 November 1998 (19.11.98)

(71) Applicant: UNIVERSITY OF CALIFORNIA [US/US]; Technology Transfer Office, Mail Code: 0093, 9500 Gilman Drive, La Jolla, CA 92093–0093 (US).

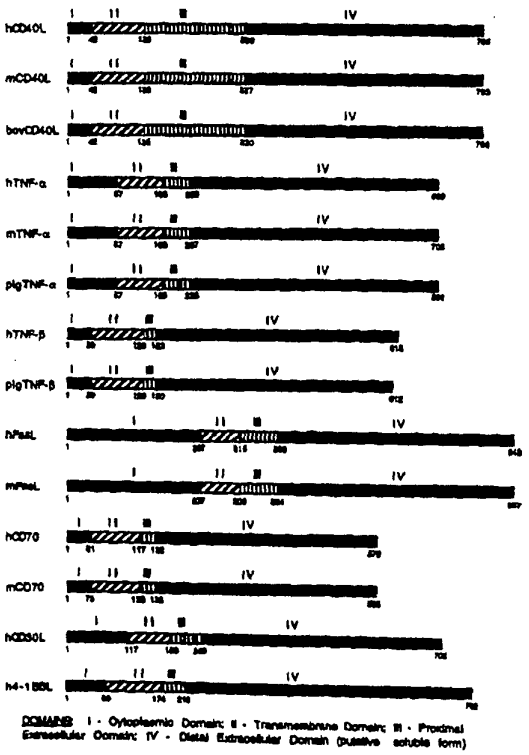
(72) Inventors: KIPPS, Thomas, J.; 661 South Nardo Road #10, Solana Beach, CA 92075 (US). SHARMA, Sanjai; 8520–K Via Mallorca, La Jolla, CA 92037 (US). CANTWELL, Mark; 3775–H Miramar Street, La Jolla, CA 92037 (US).

(74) Agents: GUISE, Jeffrey, W. et al.; Lyon & Lyon LLP, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071–2066 (US).

(54) Title: EXPRESSION VECTORS CONTAINING ACCESSORY MOLECULE LIGAND GENES AND THEIR USE FOR IMMUNOMODULATION AND TREATMENT OF MALIGNANCIES AND AUTOIMMUNE DISEASE

(57) Abstract

This invention relates to genes which encode accessory molecule ligands and their use for immunomodulation, vaccination and treatments of various human diseases, including malignancies and autoimmune diseases. This invention also describes the use of accessory molecule ligands which are made up of various domains and subdomain portions of molecules derived from the tumor necrosis factor family. The chimeric molecules of this invention contain unique properties which lead to the stabilization of their activities and thus greater usefulness in the treatment of diseases. Vectors for expressing genes which encode the molecules of this invention are also discussed.



DOMAINS: I - Cytoplasmic Domain; II - Transmembrane Domain; III - Proximal Extracellular Domain; IV - Distal Extracellular Domain (putative soluble form)

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
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AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
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DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

Figure 2.

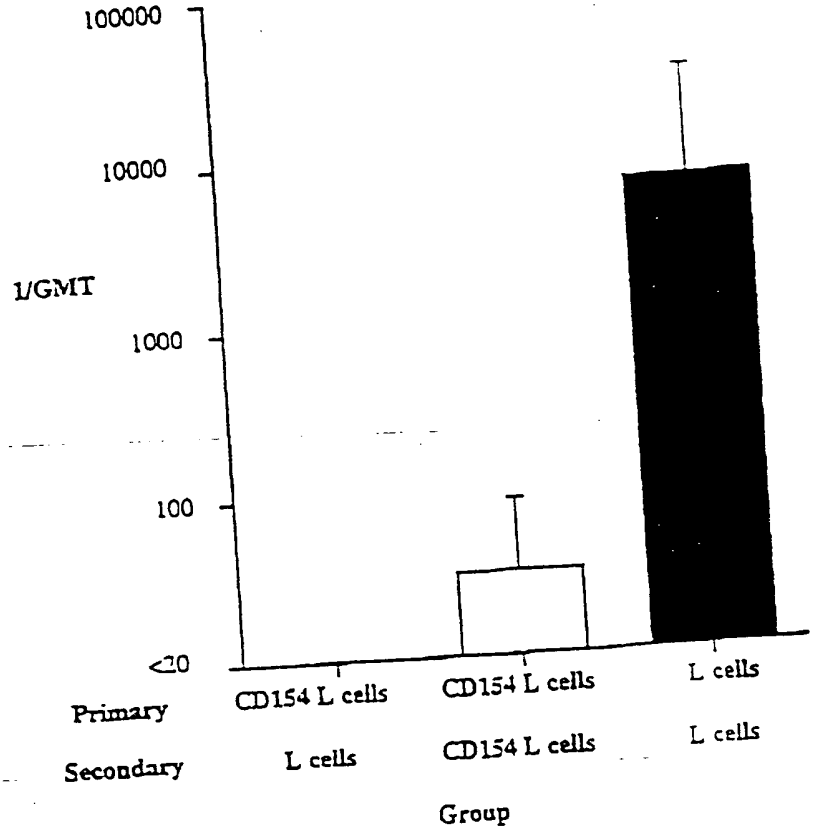
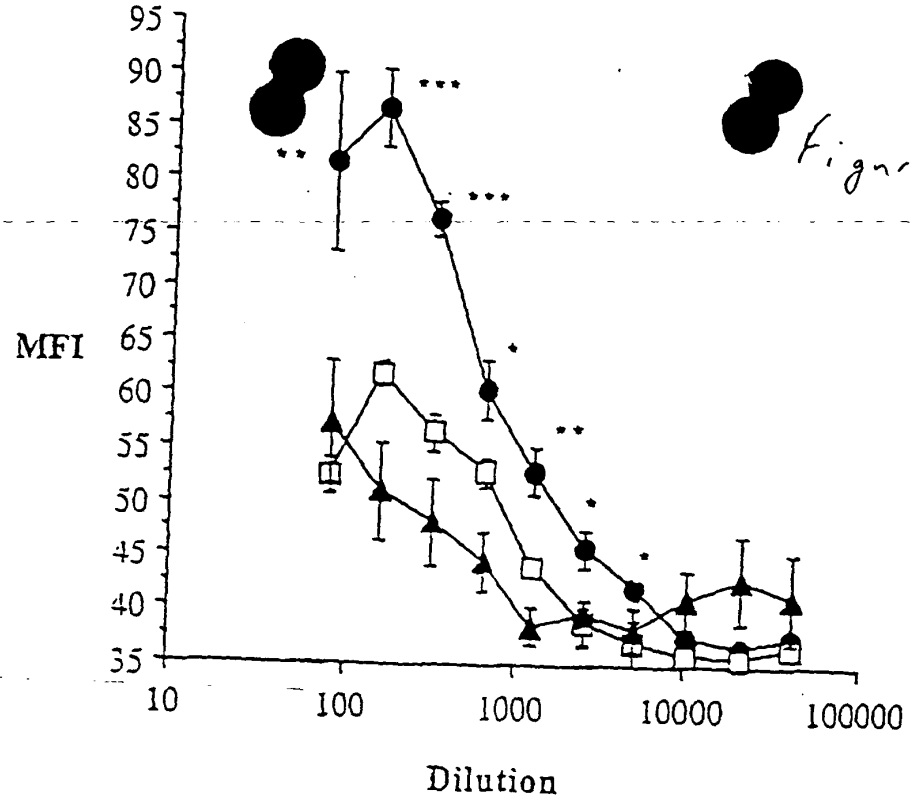

100000
10000
1/GMT
1000
100
<20
CD154 L cells
CD154 L cells
L cells
Primary
L cells
CD154 L cells
L cells
Secondary
Group

Figure 1


MFI
95
90
85
80
75
70
65
60
55
50
45
40
35
10
100
1000
10000
100000
Dilution

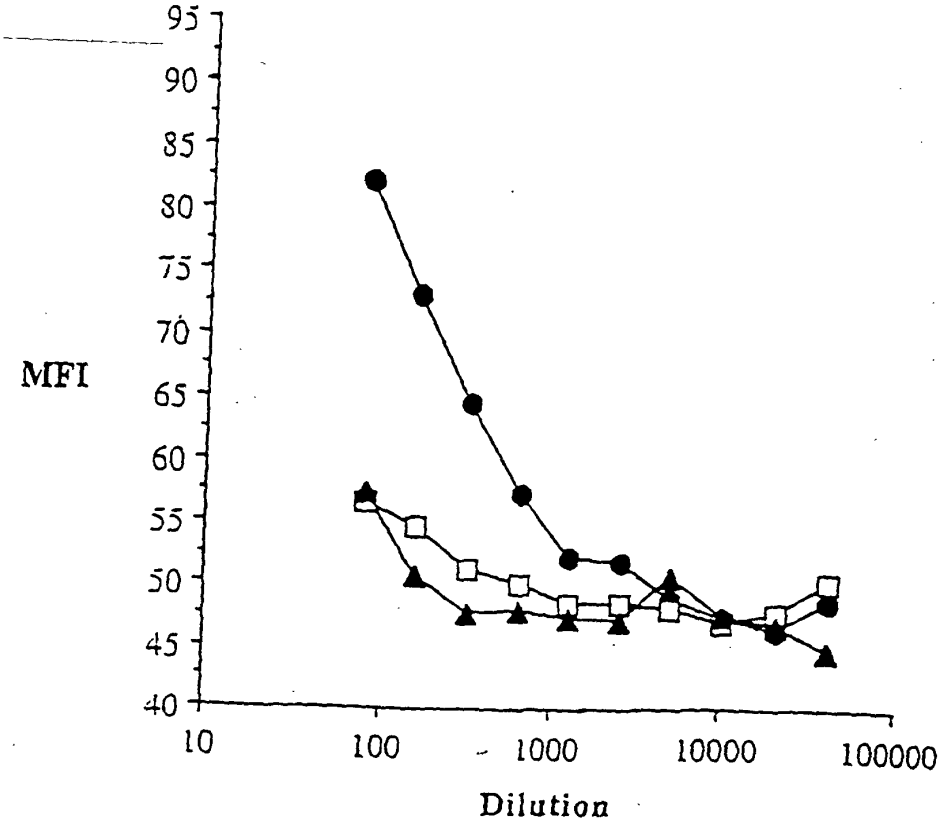
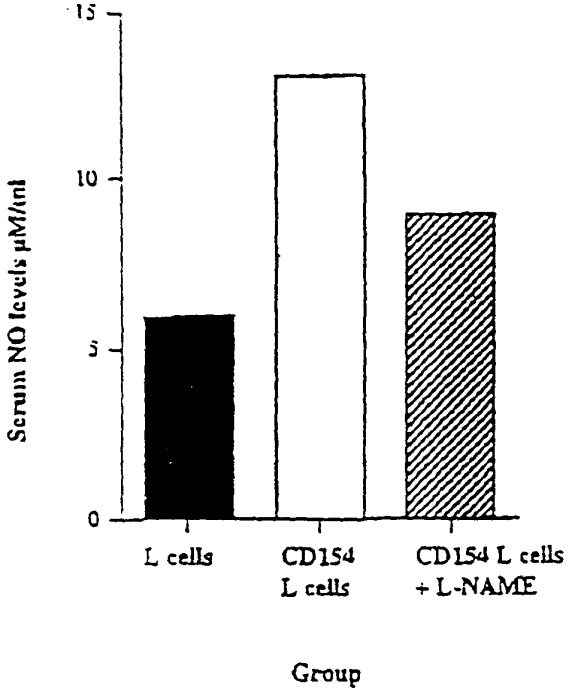
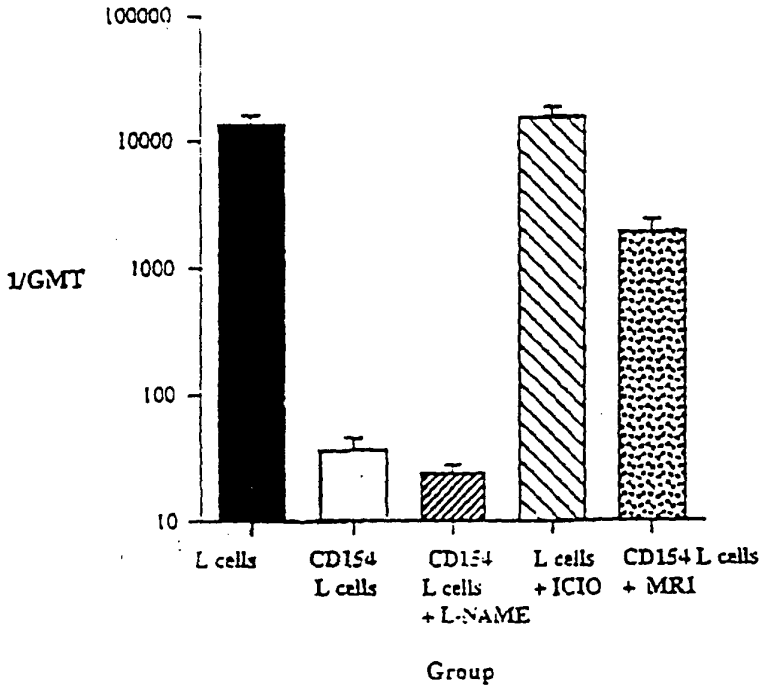

MFI
95
90
85
80
75
70
65
60
55
50
45
40
10
100
1000
10000
100000
Dilution

Figure 4


15
10
5
0
Serum NO levels μM/ml
L cells
CD154 L cells
CD154 L cells + L-NAME
Group


100000
10000
1000
100
10
1/GMT
L cells
CD154 L cells
CD154 L cells + L-NAME
L cells + ICIO
CD154 L cells + MRI
Group

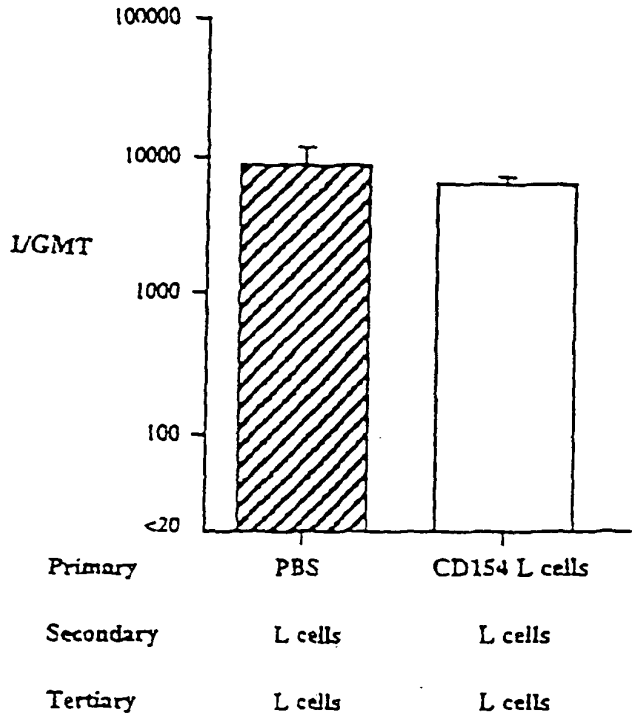

100000
10000
1/GMT
1000
100
<20
Primary
PBS
CD154 L cells
Secondary
L cells
L cells
Tertiary
L cells
L cells
Group

Figure 3

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]US 97/22740

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/62 C07K14/48 C07K14/52 C07K14/525
C07K14/705 C12N15/86 A61K48/00 A61K38/17 A61K35/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 14487 A (UNIV AUSTRALIAN ;RUBY JANET CAROLINE (AU); RAMSHAW IAN ALLISTER (A) 1 June 1995	1,4-10, 23-38, 43,44, 47,67,69
Y	See the whole document, especially the examples and claims 8 and 12. see page 4, paragraph 1 ---	3,11-19, 21,22,68
Y	WO 96 18413 A (UNIV BIRMINGHAM) 20 June 1996 see page 10, paragraph 3; claims 1-9 ---	3,11-19, 21,22,68
X	WO 95 18819 A (IMMUNEX CORP) 13 July 1995	1,4-7, 23, 28-38, 47-49
Y	see page 16, line 14 - line 29 see page 6, line 15 - line 16 ---	20

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 June 1998	16. 09. 1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Smalt, R

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/US 97/22740

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 14876 A (UNIV COLORADO ;BELLGRAU DONALD (US); DUKE RICHARD C (US); FRANZUSO) 23 May 1996 See the whole document, particularly claim 23.	20
X	WO 96 22370 A (UAB RESEARCH FOUNDATION) 25 July 1996 see page 43, line 29 - line 31	2
X	WO 94 17196 A (IMMUNEX CORP) 4 August 1994 See the whole document, especially claim 13.	46
X	EP 0 675 200 A (MOCHIDA PHARM CO LTD ;OSAKA BIOSCIENCE INST (JP)) 4 October 1995 See the whole document, especially the examples, and claim 21,40,41,43.	48,49
X	WO 93 08207 A (IMMUNEX CORP) 29 April 1993 see claims 15-17; example 13	50-56
X	WO 95 32627 A (UNIV COLORADO ;BELLGRAU DONALD (US); DUKE RICHARD C (US)) 7 December 1995 see page 19, line 22 - line 29; claims 12,16,20; example 14 see page 14, line 10	70-77
A	WO 94 04680 A (SCHERING CORP) 3 March 1994 see page 77, line 10 - line 19	9,10,15, 19,25, 27,53, 54,72, 74,75
A	CANTWELL, M.J. ET AL.: "CD95 and FAS-ligand expression and apoptosis in rheumatoid arthritis." ARTHRITIS AND RHEUMATISM, vol. 39, no. 9, suppl., September 1996, page 287 XP002066597 see the whole document	20,70-77
P,X	WIERDA, W.G. ET AL.: "Infection of B-cell lymphoma with adenovirus vector encoding CD40-ligand (CD154) induces phenotypic changes that allow for autologous immune recognition." BLOOD, vol. 90, no. 10, 15 November 1997, page 2280 XP002066598 see the whole document	3,9, 11-19, 21,28, 45,50,55 2

-/--

INTERNATIONAL SEARCH REPORT

International Application No
US 97/22740

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KATO, K. ET AL.: "Adenovirus-mediated gene transfer of CD40-ligand induces autologous immune recognition of chronic lymphocytic leukemia B cells." BLOOD, vol. 90, no. 10, 15 November 1997, page 1157 XP002066599 see the whole document -----	2,3, 11-19, 21,28,45

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ational application No.
PCT/US 97/22740

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sh et)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 11-22, 51-56, 70-77 and 1-10, and 67 in as far as they relate to in vivo methods, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see further information sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see further information sheet, subject 1.

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3,5-9,11-13,16-25,28-37,50,51,55,56,70,73,74, 77 and 4,10,14,15,26,27,38,43-49,52-54,67-69,71,72, 75

Method of altering the immunoreactivity of cells by expression of an A.M.L. (accessory molecule ligands) on the surface of these cells, method of inducing an immune response using said cells, method of treating neoplasia and/or rheumatoid arthritis using said cells, gene therapy vector and gene therapy construct encoding said A.M.L., method of vaccination using said vector, construct or cell, and cells containing said vector or construct.

2. Claims: 39-42,57-66,78-82 and 4,10,14,15,26,27,38,43-49, 52-54,67-69,71,72,75,76,83 partially

Chimeric A.M.L. and method of altering the immunoreactivity of cells by expression of said chimeric A.M.L. on the surface of these cells, method of treating neoplasia and/or rheumatoid arthritis using said cells, gene therapy vector and gene therapy construct encoding said A.M.L., method of vaccination using said vector, construct or cell, and cells containing said vector or construct.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/22740

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9514487 A	01-06-95	AU 1059095 A	13-06-95
WO 9618413 A	20-06-96	AU 691996 B	28-05-98
		AU 3988495 A	03-07-96
		CA 2207779 A	20-06-96
		EP 0806963 A	19-11-97
		FI 972484 A	12-06-97
		NO 972732 A	13-06-97
WO 9518819 A	13-07-95	AU 1563295 A	01-08-95
		CA 2179909 A	13-07-95
		EP 0739350 A	30-10-96
		JP 9508009 T	19-08-97
		NZ 279108 A	24-06-97
WO 9614876 A	23-05-96	AU 4283596 A	06-06-96
		EP 0789593 A	20-08-97
		US 5691183 A	25-11-97
WO 9622370 A	25-07-96	AU 5020196 A	07-08-96
WO 9417196 A	04-08-94	US 5565321 A	15-10-96
		AU 677995 B	15-05-97
		AU 6231394 A	15-08-94
		CA 2153806 A	04-08-94
		EP 0679191 A	02-11-95
		JP 9500001 T	07-01-97
EP 0675200 A	04-10-95	JP 8127594 A	21-05-96
		AU 689157 B	26-03-98
		AU 8115894 A	29-05-95
		CA 2153507 A	18-05-95
		WO 9513293 A	18-05-95
WO 9308207 A	29-04-93	AU 661360 B	20-07-95
		AU 3122693 A	21-05-93
		CA 2121798 A	29-04-93
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/22740

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

[redacted] tional Application No
T/US 99/19915

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K48/00 A01K67/027 //C07K14/705,C07K14/715,C12N15/62, C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 06241 A (GEN HOSPITAL CORP) 20 February 1997 (1997-02-20) page 1, line 24-33 page 6, line 24-29 claims	1,14-18, 21,34-40
A		2,4-13, 19, 23-33,41

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2000	22/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GAINER A L ET AL: "Improved survival of biolistically transfected mouse islet allografts expressing CTLA4-Ig or soluble Fas ligand." TRANSPLANTATION, (1998 JUL 27) 66 (2) 194-9. , XP000877391 abstract page 198, left-hand column, line 18-22 table 2	1,3,20, 22
A	---	2,4-13, 19, 23-33,41
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INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/US 99/19915

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 20-39 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/19915

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9706241 A	20-02-1997	AU 6687696 A	05-03-1997
		EP 0842266 A	20-05-1998
		JP 11510698 T	21-09-1999

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/02652

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 C12N5/10 A61K35/12 A61K7/00
A61L15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 26061 A (UNIV CALIFORNIA) 18 June 1998 (1998-06-18) the whole document ---	1-26
A	GAINER A. L. ET AL.: "IMPROVED SURVIVAL OF BIOLISTICALLY TRANSFECTED MOUSE ISLET ALLOGRAFTS EXPRESSING CTLA4-IG OR SOLUBLE FAS LIGAND" TRANSPLANTATION, vol. 66, no. 2, 27 July 1998 (1998-07-27), pages 194-199, XP000877391 ISSN: 0041-1337 the whole document --- -/--	1-26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2000	08/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mandl, B

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GRUSS H.-J. AND DOWER S. K.: "Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas" BLOOD, vol. 85, no. 12, 15 June 1995 (1995-06-15), pages 3378-3404, XP002094502 ISSN: 0006-4971 the whole document	1-26
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P,X	MCCORMICK A. L. ET AL.: "Cell surface expression of CD154 inhibits alloantibody responses: A mechanism for the prevention of autoimmune responses against activated T cells?" CELLULAR IMMUNOLOGY, vol. 195, no. 2, 1 August 1999 (1999-08-01), pages 157-161, XP002151134 ISSN: 0008-8749 the whole document	1-26

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/02652

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 20 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/02652

Patent document cited in search report	Publication date	Patent family member(s)	Publication dat
WO 9826061 A	18-06-1998	AU 5795798 A	03-07-1998
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		EP 0948614 A	13-10-1999
		NO 992756 A	09-08-1999
WO 0012138 A	09-03-2000	AU 5699599 A	21-03-2000

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Harrison Goddard Foote
Tower House
Merrion Way
Leeds LS2 8PA
GRANDE BRETAGNE

23.OCT.2001* 1935

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	15.10.2001
Applicant's or agent's file reference: P38044WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/02652	10/07/2000	23/07/1999

Applicant
UNIVERSITY OF SHEFFIELD et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 February 2001 (01.02.2001)

PCT

(10) International Publication Number
WO 01/07605 A1

(51) International Patent Classification[7]: C12N 15/12, C07K 14/705, C12N 5/10, A61K 35/12, 7/00, A61L 15/00

(21) International Application Number: PCT/GB00/02652

(22) International Filing Date: 10 July 2000 (10.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9917180.3 23 July 1999 (23.07.1999) GB

(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF SHEFFIELD** [GB/GB]; Western Bank, Sheffield S10 2TN (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **HEATH, Andrew, William** [GB/GB]; University of Sheffield, Medical School, Sheffield S10 2RX (GB).

(74) Agent: **HARRISON GODDARD FOOTE**; Tower House, Merrion Way, Leeds LS2 8PA (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/07605 A1

(54) Title: CD154 LIGAND, AND RECOMBINANT CELLS EXPRESSING IT

(57) Abstract: The invention relates to cells and/or tissues and/or organs which do not naturally express the cell surface receptor CD40 ligand, CD154, for use in therapeutic and cosmetic tissue engineering and/or organ transplantation.

CD154 LIGAND, AND RECOMBINANT CELLS EXPRESSING IT

The invention herein described relates to cells and/or tissues and/or organs which do not naturally express the cell surface receptor CD40 ligand, CD154, for use, particularly but not exclusively, in therapeutic and cosmetic tissue engineering and/or organ transplantation; compositions comprising said cells and/or tissues; organs comprising said cells/tissues; and methods of therapy and/or cosmetic surgery using said cells and/or tissues and/or organs.

Tissue engineering is an emerging science which has implications with respect to many areas of clinical and cosmetic surgery. More particularly, tissue engineering relates to the replacement and/or restoration and/or repair of damaged and/or diseased tissues to return the tissue and/or organ to a functional state. For example, and not by way of limitation, tissue engineering is useful in the provision of skin grafts to repair wounds occurring as a consequence of: contusions, or burns, or failure of tissue to heal due to venous or diabetic ulcers. Further, tissue engineering is also practised during: replacement of joints through degenerative diseases such as arthritis; replacement of coronary arteries due to damage as a consequence of various environmental causes (e.g. smoking, diet) and/or congenital heart disease including replacement of arterial/heart valve; repair of gastric ulcers; replacement bone tissue resulting from diseases such as osteoporosis; replacement muscle and nerves as a consequence of neuromuscular disease or damage through injury.

In addition, organ transplantation has for many years been an established surgical technique to replace damaged and/or diseased organs. The replacement of heart, lung, kidney, liver, bone marrow, and double organ transplantation of, for example and not by way of limtation, heart and lung, are relatively common procedures.

However, in both tissue engineering and organ transplantation a major obstacle to the successful establishment of a tissue graft or organ transplantation is the host's rejection of the donated tissue or organ.

With respect to tissue engineering and organ transplantation, surgeons currently have three types of graft/organ:

i. an autograft in which a piece of tissue is removed from one area of a patient's body and placed in another location;

ii. an allograft, in which a section of tissue from one human, for example a cadaver, is grafted onto another human; and

iii. a xenograft, where tissue is harvested from another species, for example a pig, and placed over the wound area.

Autografts can be problematic due to the availability of suitable tissue and the added trauma to the patient following the removal of the tissue from another part of the body to the wound area. Allografts can be problematic due to the immunological reactivity of the host and/or the availability of donor tissue/organ. Xenografts are even more problematic due to the severe immunological reactivity of the host and the psychological problems relating to the implantation or grafting of tissue/organ from a non- human species onto or into a human body.

The body has developed many defences against invasion of foreign organisms. These humoral and cellular defence mechanisms are also directed against foreign antigens expressed by various tissues/organs used in tissue engineering and/or organ transplantation.

A general term to cover a number of distinct cell types intimately involved in both a humoral and cellular defence mechanism is white blood cells. Each white blood cell type has a separate role to play in a hosts immune system. Monocytes are large white blood cells that differentiate into macrophages. The macrophages are found throughout the body in various types. For example specialised macrophages include alveolar macrophages in the lungs, mesangial phagocytes in the kidneys, microglial cells in the brain, and Kuppfer cells in the liver. Macrophages have many roles and

these include, by example and not by way of limitation, ingestion of infectious agents, antigen presentation to T-lymphocytes and the secretion of agents involved in regulating the immune system (i.e. interleukin-1, complement proteins).

A pivotal cell-cell interaction between the many cell types of the immune system is between T- lymphocytes (T- cells) and B- lymphocytes (B- cells). T – cells recognise polypeptide antigens presented as peptides via self molecules referred to as the major histocompatibility complex (MHC) on antigen presenting cells such as macrophages. T-cells are divided into cytotoxic T- cells (CTL's) and T- helper cells. The latter class of T-cell are able to stimulate B- cell proliferation and mediate immunoglogulin isotype switching to produce antibody isotypes (IgG, IgA, IgD, IgM, IgE) to specific peptide antigens.

The regulation of biochemical and physiological responses to foreign antigens is, by and large, mediated through intercellular and/or intracellular receptor mediated activation via ligand binding. Typically, ligands which interact with receptors to bring about a suitable biochemical/metabolic response are known as agonists and those that prevent, or hinder, a biochemical/metabolic response are known as antagonists.

The interaction of T helper cells and B-cells involves receptor/ligand binding. The B-cell CD40 receptor (CD40 is a monoclonal antibody which recognises the receptor) and the T-cell ligand gp39, referred to hereinafter as CD154 (CD 154 is a monoclonal antibody which recognises gp39) interact and play a pivotal role in both the humoral and cellular immune responses to T- cell dependent (TD antigens). In the absence of either molecule there is no isotype switching in response to a T cell dependent antigen, no germinal centre formation, and no enhanced secondary antibody responses (1,2)

The human CD40 receptor is a 48kDa, 277 amino acid polypeptide, transmembrane glycoprotein expressed predominantly at the B- cell surface. This receptor is also expressed by a number of other cell types. For example and not by way of limitation,

monocytes, basophils, eosinophils, endothelial cells, Langerhans cells, keratinocytes, Kaposi's sarcoma cells.

The human CD154 is a 33kDa, 261 amino acid polypeptide, transmembrane glycoprotein predominantly expressed at the T cell surface. This ligand is also expressed by a number of other cell types. For example, and not by way of limitation, mast cells, basophils, eosinophils, dendritic cells and monocytes. It is of note that CD154 has not been shown to be expressed in somatic cells other than those which are closely associated with the immune system.

Given the importance of the interaction of CD40 receptor with CD154 we have undertaken a study of the interaction between these molecules by expressing CD154 in cells which do not naturally express CD154, namely mouse fibroblasts expressing a mismatched MHC class, and used these cells as an immunogen. We anticipated that this would act as a potent stimulator of the immune system.

It is known that blocking the interaction of CD40 with CD154 can suppress the immune response. For example, the use of anti- CD154 antibodies is known to abrogate the interaction between CD40 receptor and CD154 and result in attenuation of the immune system in response to allografts (WO9856417 & WO9858669); suppression of autoimmune disease (WO9900143) and blood clotting disorders (WO9858672). We predicted that the recombinant expression of CD154 in MHC mismatched cells would promote an immune response to said cells. To our surprise, these cells did not promote an immune response but resulted in immune suppression toward the injected transfected fibroblasts.

It is apparent that this observation has important implications with respect to allotypic recognition of implanted cells/tissues/organs. The expression of CD154 in cell types which do not naturally express this ligand resulted in failure of the immune system to recognise the implanted cells as foreign. This has important implications with respect to tissue/organ transplantation and tissue/organ rejection by the host receiving the transplanted tissue/organ.

According to a first aspect of the invention there is provided at least one cell/tissue/organ for use in tissue engineering and/or organ transplantation wherein said cell/tissue/organ does not naturally express the CD154 ligand but is adapted to express at least an effective part of the CD154 ligand.

Reference herein to a part of CD154 includes reference to at least part of the extracellular domain.

In a preferred embodiment of the invention said cell/tissue/organ is transfected with DNA encoding at least the effective part of CD154, or a homologue thereof.

In yet a further preferred embodiment of the invention said DNA is genomic DNA.

In yet still a further preferred embodiment of the invention said DNA is cDNA.

In yet a still further preferred embodiment of the invention said cells/tissues/organ are of mammalian origin. Ideally said cells/tissues/organs are of human origin.

In yet still a further preferred embodiment of the invention said cells/tissues are selected from the following cell types: fibroblast; keratinocyte; osteoblast; chondrocyte; neurones, myocytes; hepatocytes; splenocytes, pancreatic β cells.

According to a second aspect of the invention there is provided a vector for use in the transfection of a selected cell/tissue/organ type for use in tissue engineering and/or organ transplantation characterised in that it contains a DNA molecule encoding at least an effective part of CD154 ligand, or a homologue thereof.

In a preferred embodiment of the invention said vector is adapted for the recombinant expression of CD154 ligand.

Conventionally, nucleic acid molecules used to transfect cells are referred to as vectors. Vectors used in genetic engineering are typically circular molecules, (although some may be linearised prior to transfection to facilitate the introduction of DNA into a host cell). Vectors of this type are referred to as plasmids, phages, or phagemids. In many examples these vectors have been genetically engineered to adapt them for expression in eukaryotic cells. For example, and not by way of limitation the provision of cell/tissue specific promoter elements which facilitate expression in a specific cell/tissue type; the provision of viral promoters which provide high levels of constitutive expression.

In addition to the above identified vectors, viral based vectors are used in transfection and in particular, gene therapy, to deliver genes to tissues *in vivo*. These vectors typically retain the capability to infect a host cell but are genetically modified to render the virus biologically disabled; this latter feature facilitates its removal from the organism and prevents its uncontrolled spread through host tissues. Examples of viral based vectors used in gene therapy include by example and not by way of limitation: adenovirus; retrovirus; parvovirus; and herpesvirus

In a further preferred embodiment of the invention said adaptation comprises the inclusion of appropriate expression control sequences which optimise the expression of the vector encoded nucleic acid molecule.

It will be apparent to one skilled in the art that said adaptation relates to a vector adapted for expression in a eukaryotic cell. For example, and not by way of limitation, said adaptation comprises the provision of constitutive, inducible, or repressible promoter elements; and/or the provision of polyadenylation control sequences for optimal expression; and/or the provision of selectable markers to allow the selection of said vector in a eukaryotic cell.

Furthermore the provision of regulatable promoter elements to control the amount of CD154 available to the cell/tissue is advantageous. It is desirable to regulate the amount of CD154 in accordance with the immune status of the host. For example,

once the cell/tissue/organ has been implanted it is may be beneficial to reduce the amount of CD154 expressed as the host becomes tolerant of the transplanted cell/tissue/organ. Alternatively, CD154 expression can be increased if the host begins to reject the donated cells/tissue/organ. This can be facilitated by modulation of the regulatable promoter to increase or decrease the amount of CD154.

In yet a further preferred embodiment of the invention there is provided a vector comprising a cell/tissue specific promoter sequence for use in the cell/tissue specific expression of CD154 according to any previous aspect or embodiment of the invention.

In a preferred embodiment of the invention said cell/tissue/organ is selected from the following tissue types: neuronal, muscle (e.g. smooth, striated, cardiac), bone, cartilage, liver, kidney, respiratory epithelium, endothelium, haematopoietic cells, spleen, pancreas, skin, stomach, intestine, oesophagus;blood vessels.

According to a third aspect of the invention there is provided a method to transfect a selected cell/tissue comprising:

i. providing a vector according to the invention and adding said vector to cells/tissues;

ii. incubating cells/tissues under conditions conducive to the introduction and maintenance of a vector;

iii. exposing said cells to an agent at a concentration sufficient such that at least those cells/tissues including said vector are selected for; and optionally,

iv. culturing said cells/tissues containing said nucleic acid molecule and, optionally, further still,

v. storing said cell culture prior to use.

In a preferred method of the invention said cell/tissue is a mammalian cell/tissue. Ideally said mammalian cell/tissue is of human origin.

In a further preferred method of the invention, said transfection is transient. In the event that a transient transfection is required; steps (ii) and (iii) are not necessary.

It will be apparent to a man skilled in the art that where steps (ii) and (iii) are undertaken stable transfection is facilitated

Eukaryotic cells may be transfected via a variety of techniques. For example, and not by way of limitation, DNA may be introduced into mammalian cells via calcium phosphate precipitation (Graham, FL and Van der Eb AJ, (1973) Virology 52, p456). This technique is particularly useful for both transient and stable transfection. An alternative to calcium phosphate precipitation is DEAE dextran mediated transfection (Gluzman, Y. (1981) Cell, 23, 175). This method is used primarily for transient transfection rather than stable transfection.

More recently, eukaryotic cells have been transfected using a pulse of high voltage electricity which, when passed through a culture of cells in the presence of vector DNA, momentarily results in permeabilisation of the cell membrane thus facilitating the introduction of vectors into said cells. This procedure is referred to as electroporation. Furthermore, an alternative to the above mentioned methods is so called "ballistic" transfection where DNA coated microbeads are "shot" into cells/tissues to deposit the DNA into the cell/tissue.

According to a fourth aspect of the invention there is provided a therapeutic composition comprising at least one cell/tissue according to any previous aspect or embodiment of the invention.

Preferably, said therapeutic composition is adapted for use in tissue engineering. More preferably still, said tissue engineering is the replacement of diseased or damaged tissue.

Conditions which would benefit from therapeutic tissue engineering include by example, and not by way of limitation, arthritis and the replacement of joints; skin grafting for burn victims or injuries resulting in sever contusions; replacement of coronary arteries; replacement of diseased or damaged nerves and/or muscles; replacement of pancreatic β cells.

According to a fifth aspect of the invention there is provided at least one organ wherein said organ for use in organ transplantation comprises at least one cell/tissue according to any previous aspect or embodiment of the invention.

In a preferred embodiment of the invention said organ comprises at least one cell/tissue transfected with the vector according to any previous aspect or embodiment of the invention.

According to a sixth aspect of the invention there is provided a cell/tissue composition for use in cosmetic tissue engineering comprising at least one cell/tissue according to any previous aspect or embodiment of the invention.

In a preferred embodiment of the invention said cell/tissue comprises at least one cell/tissue transfected with the vector according to any previous aspect or embodiment of the invention.

According to an seventh aspect of the invention there is provided a method of treatment comprising;

i) providing at least one cell/tissue which does not normally express CD154, or effective part thereof, and adapted so that same expresses at least the effective part of CD154;

ii) administering said cells/tissues to a patient to be treated; and optionally,

iii) monitoring the status of said cells/tissues by the patient.

According to an eighth aspect of the invention there is provided a method of treatment comprising;

iv) providing at least one organ which does not normally express CD154, or effective part thereof, comprising at least one cell/tissue expressing at least the effective part of CD154;

v) surgical implantation of said organ to a patient to be treated; and optionally

vi) monitoring the status of said cells/tissues by the patient.

When said cell/tissue/organ comprises an inducible promoter whereby CD154 may be selectively expressed said method may further involve monitoring/regulating the administration of at least one agent that activates said promoter.

In a preferred method of the invention said cell/tissue/organ comprises at least one cell transfected with a nucleic acid molecule encoding at least the effective part of CD154 according to any previous aspect or embodiment.

According to a further aspect of the invention there is provided a vehicle wherein said vehicle has least one cell according to the invention attached thereto.

Vehicle is defined as any structure to which cells according to the invention may attach and proliferate. For example and not by way of limitation, a prosthesis, implant, matrix, stent, gauze, bandage, plaster, biodegradable matrix and polymeric film.

In a preferred embodiment of the invention there is provided a therapeutic vehicle comprising cells according to the invention wherein said therapeutic vehicle is adapted to be applied and/or implanted into a patient requiring therapeutic tissue engineering.

In yet a further preferred embodiment of the invention there is provided a therapeutic vehicle comprising a matrix material (for example, and not by way of limitation, a

matrix material which is synthetic or naturally occuring and either long-lasting or biodegradable) comprising at least one cell according to the invention for use in surgical implantation procedures.

According to a yet further aspect of the invention there is provided a cosmetic vehicle comprising at least one cell according to the invention for use in cosmetic tissue engineering.

An embodiment of the invention will know be described, by example only, and with reference to the following Figures;

Figure 1 is a representation of groups of five BALB/c mice immunised twice intraperitoneally with 5 x 10^4 L cells (closed circles), CD154 L cells (open squares), or PBS (closed triangles);

Figure 2 represents groups of five BALB/c mice immunised with L cells or CD154 L cells and boosted with either L cells or CD154 L cells;

Figure 3 represents groups of five BALB/c mice immunised with CD154 L cells or PBS, immunised again with normal L cells 3 and 6 weeks later and bled 8 days after the last immunisation;and

Figure 4 represents measurements of serum nitrate levels of mice after immunisation with L cells or CD154 L cells.

MATERIALS AND METHODS

Cells and antibodies

L929 cells (L cells) and CD154 transfected L929 cells (CD154 L cells) were kindly provided by DNAX Research Institute, California. CD154 transfected L929 cells

were prepared as described elsewhere [15]. Anti-CD40 antibody 1C10 (9) was purified on a protein G column from hybridoma supernatant produced in a bioreactor by Sheffield hybridomas, Sheffield. The MR1 anti-CD154 mAb was purchased from Pharmingen.

Mice and Immunisations

BALB/c female mice of 8-12 weeks of age were obtained from the University of Sheffield Field Laboratories. L929 cells were removed from tissue culture flasks using EDTA (0.5mM), washed in PBS and 5 x 10^4 cells injected intraperitoneally into MHC mismatched BALB/c mice. Mice were bled 10 days post-primary immunisation and seven days after each subsequent immunisation.

Measurement of antibody responses

Antibody responses to L929 cell surface antigens were determined by Flow cytometric analysis. L929 cells, at 10^6 cells/ml, were incubated in FACS buffer (PBS, 3% BSA, 0.01% Sodium azide) at 4°C for 20 minutes with serial dilutions of mouse antisera for 20 minutes. Cells were then washed three times with FACS buffer and incubated with a FITC labelled goat anti-mouse immunoglobulins (Pharmingen) for 20 minutes at 1:100, washed three times and analysed using a Becton Dickinson FACScan analyser and "Facscan™" and "Lysis™" software. Dead cells were gated out by forward and 90° angle light scatter. Mean fluorescence intensities (MFI) were plotted against dilution and examples of such plots are shown in figure 1. To simplify the remaining figures mean endpoint titres are shown, and were determined by the points of intersection of the MFI curves with those of normal mouse serum (i.e the PBS group from figure 1). Statistical analyses were by Student's t test.

Assay for Nitrate as a measure of Nitric oxide production

Sera were assayed for Nitrite levels (as an indicator of Nitric oxide production) 2h post injection with L cells or CD154 L cells by the addition of 50µl test serum to 100µl Greiss reagent (0.5% Sulfanilamide (w/v) 0.05 % Napthylethylene (w/v) , 1.125% phosporic acid). Sodium nitrite standards were double diluted across the plate starting at 100mM. The plates were incubated at RT for 10min and absorbance at 540nm measured.

RESULTS

Expression of CD154 on antigenic cells inhibits the alloantibody response

The antibody responses of BALB/c mice against normal or CD154 expressing L929 cells were not detectable by this flow cytometric assay following a single immunisation. However two i.p injections of L cells gave rise to antibody responses against L cells of around 1/1000 (Figure 1). In contrast mice immunised with CD154 L cells produced no detectable response against L cells even after two immunisations (Figure 1). We considered it possible that these apparent differences in immunogenicity were caused by antigenic variation between the two cell lines as responses were assayed against normal L cells; however similar results were seen when CD154 L cells were used as the antigen in the assay, Figure. Thus the results were due to a lack of response to the CD154 L cells in immunised mice.

CD154 L cells fail to prime for an alloantibody response against normal L cells, but do not induce tolerance.

Mice immunised first with CD154 L cells, and then with normal L cells failed to produce a normal secondary antibody response against the L cells, thus CD154 expression inhibited priming of the antibody response against L cells ($p=0.013$; Fig

2). It was possible that the CD154 L cells were inducing tolerance, leading to a lack of response on secondary exposure to normal L cells. To determine whether this was the case, two groups of mice were immunised with two doses of L cells, but one of the groups had previously been immunised with CD154 L cells. Had the CD154 expressing cells induced tolerance then the latter group should have produced a lower immune response. In fact this was not the case, and responses between the two groups were the same ($p=0.23$; Fig 3). Thus it would appear that CD154 expression does not result in tolerance, but rather a lack of recognition of the antigen on initial exposure.

Induction of Nitric oxide by CD154 expressing L929 cells

As CD40 ligation had been shown to enhance production of NO by macrophages [7] and NO production is responsible for a generalised immunosuppression seen in acute bacterial infection [8], we investigated whether NO levels were increased in mice immunised with CD154 L cells. As shown in figure 4a, there was an increase in serum nitrate, indicating a rise in NO levels in vivo following immunisation with CD154 expressing cells which was greater than that seen after immunisation with normal L929 cells. It does not appear however that NO is responsible for the suppression of immune responses against CD154 L cells, as co-injection with the NO synthase inhibitor, L-NAME abrogated the nitric oxide production but had no effect on suppression (Fig 4b)

The suppressive effect of CD154 expression is probably not related to the particulate form of the antigen

We have shown strong positive effects of CD40 ligation on immune responses to soluble antigens using anti-CD40 antibodies (6). However when the agonistic anti-mouse CD40 antibody 1C10 [9] (500μg) was co-injected with normal L929 cells, the antibody response was neither suppressed nor significantly enhanced($p=0.33$; fig 4b). The suppression mediated by CD154 expression is therefore unlikely to be related simply to the form of the antigen, administered as whole cells rather than soluble protein

Reversal of inhibition with anti-CD154

The inhibition of alloantibody responses by CD154 expression is clearly reversed by pre-incubation of the cells with 10μg/ml MR1 (anti-CD154, 10) antibody ($p=0.0013$), again indicating the suppression of responses by these cells is due to CD154 expression and not antigenic or other differences between these cells and normal L cells (Figure 4b).

DISCUSSION

Interactions between CD154 and CD40 play a very important role in immune responses. In general, stimulation of B cells through CD40 induces strong B cell activation, proliferation and isotype switching especially in co-operation with signalling by other factors such as antigen (or anti-IgM), and cytokines such as IL4. Signalling through CD40 also appears to be important in initiating and maintaining germinal centres [1]. Activation of B cells through anti-CD40 antibodies in vivo can also give rise to enhanced isotype switching, greatly increased antibody responses [6,11] and B cell proliferation [Dullforce, Greenwood and Heath, in preparation]. As we had demonstrated strong adjuvant-like effects of CD40 ligation on antibody responses *in vivo*, we considered that cell-surface expression of the CD40 ligand, CD154, may be a potent means of enhancing anti-cellular immune responses. An analogous approach had been used successfully utilising CD80 and CD86 transfection to enhance the CTL response against tumour cells in vivo [12]. We therefore examined the effect of transfection with CD154 on the alloantibody response to murine L929 cells.

BALB/c mice ($H\text{-}2^d$) were immunised with the MHC mismatched cell line L929, untransfected or stably transfected and expressing murine CD154 on the membrane. Contrary to our expectations we found that expression of CD154, rather than enhancing the alloantibody response to the L929 cells, actually suppressed the response. This suppression only became evident after two immunisations because of

the poor primary response to normal L cells. However, the suppressive effect was mediated at the primary immunisation as the response to a second injection with normal L cells was suppressed by primary immunisation with CD154 L cells. The lack of responsiveness to CD154 L cells was not caused by antigenic differences between the two cell lines, as similar results were obtained when CD154 L cells were used as antigen to detect the antibody. It appeared possible that the CD154 expression was rendering the L cells tolerogenic. However, that did not appear to be the case as mice immunised with CD154 L cells, followed by two doses of L cells, responded normally to the second dose of L cells. It appears, therefore, as though the expression of CD154 by L cells simply prevents or suppresses effective priming or activation of the allospecific cells rather than induces tolerance.

Earlier experiments on the in vivo effects of CD40 ligation have been performed using soluble antigens and soluble anti-CD40 antibody [ ]. It was therefore important to determine whether the form of the antigen was the important factor in this apparent reversal of the effects of CD40 ligation. To this end mice were immunised with L cells and an agonistic anti-mouse CD40 antibody, 1C10 [ ] In this case the antibody response to the L cells was enhanced. Thus it would appear that the suppressive effect of CD154 expression on the L cells is dependent on: the extent of CD40 cross-linking; the temporal differences between that cross linking by a cell surface antigen and that of soluble antibody; or qualitative differences between antibody and ligand induced CD40 signalling.

Of course immune responses to allogeneic cells are different in many ways to immune responses against soluble antigens, not least in that the antigen itself is probably directly recognised by T cells without processing by a specialised APC. From the perspective of the B cell, encounter with a CD154 expressing L cell must be similar to an encounter with an activated T cell which may well also be expressing antigens which do not appear during B cell maturation, thus not allowing elimination of B cells reactive with these antigens in the bone marrow. One of these would be the T cell receptor idiotype, and another may well be CD154. It has been proposed that autoimmune responses against newly expressed antigens (such as hormones and

breast milk) do not normally occur because of the lack of a "danger" signal. There is little evidence for CD154 expression in the bone marrow microenvironment, thus B cells expressing surface immunoglobulin specific for CD154 as well as TCR idiotopes might be expected to be present in the periphery. Because of the function of CD154 , a CD154 specific B cell coming across it for the first time would receive a potent "danger" signal in the form of CD40 ligation. Thus it might be expected that strong autoantibody responses against CD154 would be produced. This is clearly not the case, and we propose that simultaneous and long-lived stimulation through surface immunoglobulin and CD40 and/or extensive cross-linking of the two receptors, may anergise the B cell preventing the production of large amounts of autoantibody against CD154 and possibly TCR idiotopes.

We consider that transfection of donor cells for transplantation with CD154 will have some role to play in enhancing the acceptance of allografts by the recipient.

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CLAIMS

1. A cell wherein said cell does not naturally express the CD154 ligand but is adapted to express CD154 .

2. A cell according to claim 1 wherein said cell is transfected with DNA encoding at least the effective part of the CD154 ligand, or homologue thereof.

3. A cell according to claim 2 wherein said cell is transfected with genomic DNA.

4. A cell according to claim 2 wherein said cell is transfected with cDNA.

5. A cell according to any of claims 1 - 4 wherein said cell is of mammalian origin.

6. A cell according to claim 5 wherein said cell is of human origin.

7. A cell according to any of claims 1 - 6 wherein said cell is selected from the following cell types: fibroblast; keratinocyte; osteoblast; chondrocyte; neurone; myocyte; hepatocyte; splenocyte; pancreatic β cells.

8. A vector for use in the transfection of a cell characterised in that said vector contains a DNA molecule encoding at least the effective part of the CD154 ligand, or homologue thereof.

9. A vector according to claim 8 wherein said vector is adapted for recombinant expression of the CD154 ligand.

10. A vector according to claim 9 wherein said adaptation comprises the inclusion of appropriate expression control sequences which optimise the expression of the vector encoded nucleic acid molecule.

11. A vector according to claim 9 or 10 wherein said vector comprises a cell/tissue specific promoter sequence for use in the cell/tissue specific expression of the CD154 ligand according to any of claims 2 - 10.

12. A vector according to claim 11 wherein said promoter is cell/tissue specific for one of the following tissue types: neuronal; smooth muscle; striated muscle; cardiac muscle; bone; cartilage; liver; kidney; respiratory epithelium; endothelium; haematopoietic cells; spleen; pancreas; skin; stomach; intestine; oesophagus; blood vessels.

13. A method to transfect a selected cell/tissue comprising;

i) incubating cells/tissues under conditions conducive to the introduction and maintenance of a vector according to any of claims 8 - 12;

ii) exposing said cells to an agent at a concentration sufficient such that at least those cells/tissues including said vector are selected for and optionally,

iii) culturing said cells/tissues containing vector; and, optionally further still,

iv) storing said cell culture prior to use.

14. A method according to claim 13 wherein said cell/tissue is a mammalian cell/tissue.

15. A method according to claim 13 or 14 wherein said mammalian cell/tissue is of human origin.

16. A method according to any of claims 13 - 15 wherein said transfection is transient.

17. A therapeutic composition comprising a cell according to any of claims 1-7.

18. An organ wherein said organ comprises at least one cell according to claim 1.

19. An organ wherein said organ comprises at least one cell transfected with the vector according to any of claims 8-12.

20. A method of treatment comprising:

i. providing at least one cell which does not naturally express the CD154 ligand, or the effective part thereof; and

ii. administering said cell to a patient to be treated.

21. A therapeutic composition comprising a cell according to any of claims 1-7.

22. Use of a cell according to any of claims 1-7 for use in the manufacture of a medicament for use in therapeutic tissue engineering.

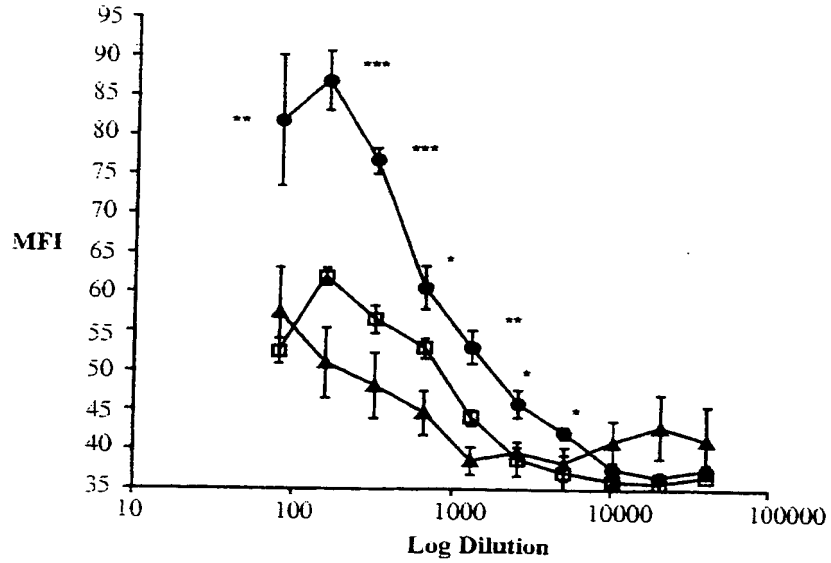
23. Use of a cell according to any of claims 1-7 for use in the manufacture of a medicament for use in cosmetic tissue engineering.

24. A therapeutic vehicle comprising a cell according to any of claims 1-7.

25. A therapeutic vehicle according to claim 24 wherein said therapeutic vehicle is selected from: a prosthesis; implant; matrix; stent; gauze; bandage; plaster; biodegradable matrix; polymeric film.

26. A cosmetic vehicle comprising a cell according to any of claims 1-7.

Figure 1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80
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70
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Log Dilution
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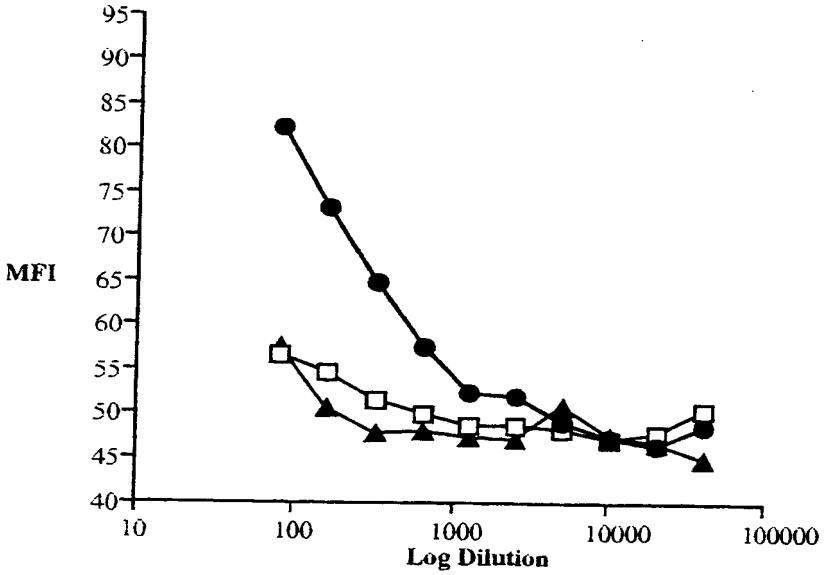
MFI
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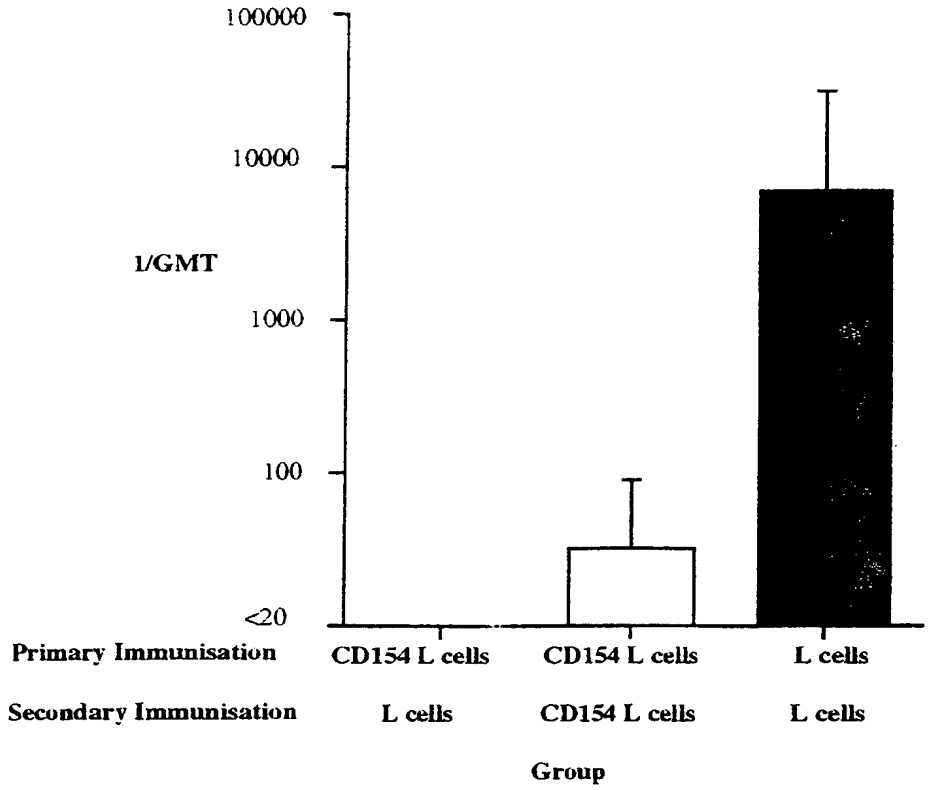
Log Dilution

Figure 2


100000
10000
1/GMT
1000
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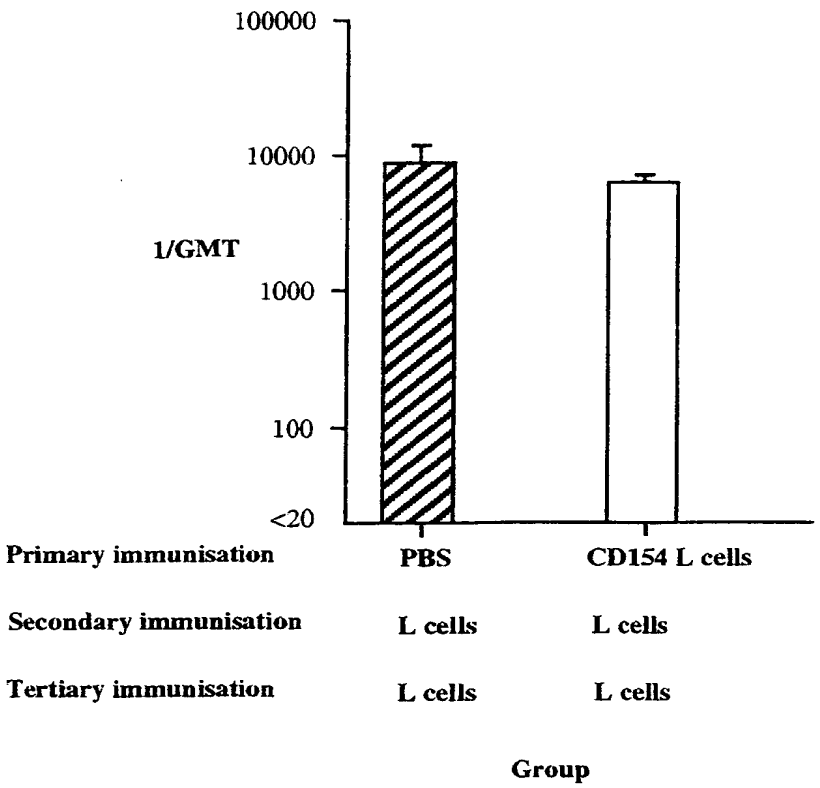
Primary Immunisation
CD154 L cells
CD154 L cells
L cells
Secondary Immunisation
L cells
CD154 L cells
L cells
Group

Figure 3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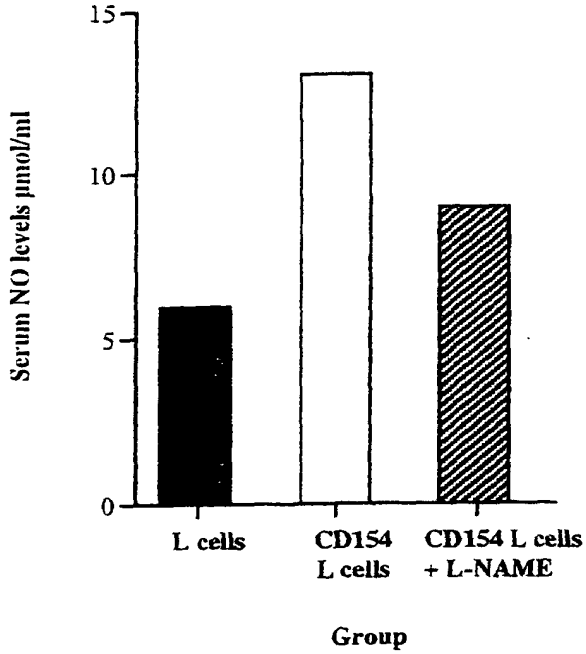
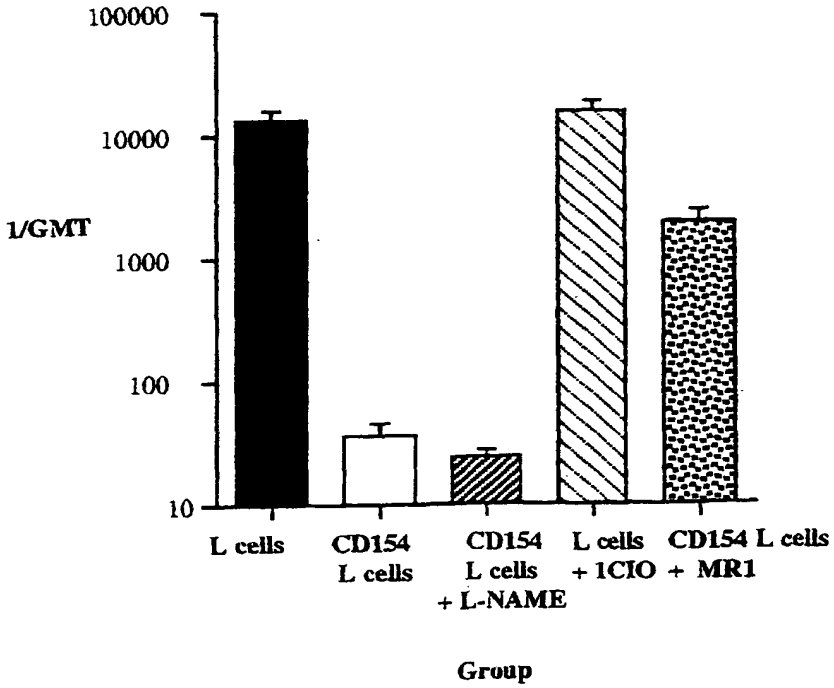
PBS
CD154 L cells
Secondary immunisation
L cells
L cells
Tertiary immunisation
L cells
L cells
Group

Figure 4


15
10
5
0
Serum NO levels µmol/ml
L cells
CD154 L cells
CD154 L cells + L-NAME
Group


100000
10000
1000
100
10
1/GMT
L cells
CD154 L cells
CD154 L cells + L-NAME
L cells + 1CIO
CD154 L cells + MR1
Group

INTERNATIONAL SEARCH REPORT

nal Application No
GB 00/02652

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 C12N5/10 A61K35/12 A61K7/00 A61L15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 26061 A (UNIV CALIFORNIA) 18 June 1998 (1998-06-18) the whole document ---	1-26
A	GAINER A. L. ET AL.: "IMPROVED SURVIVAL OF BIOLISTICALLY TRANSFECTED MOUSE ISLET ALLOGRAFTS EXPRESSING CTLA4-IG OR SOLUBLE FAS LIGAND" TRANSPLANTATION, vol. 66, no. 2, 27 July 1998 (1998-07-27), pages 194-199, XP000877391 ISSN: 0041-1337 the whole document --- -/--	1-26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2000	08/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mandl, B

1

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GRUSS H.-J. AND DOWER S. K.: "Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas" BLOOD, vol. 85, no. 12, 15 June 1995 (1995-06-15), pages 3378-3404, XP002094502 ISSN: 0006-4971 the whole document	1-26
A	EDGE A. S. B. ET AL.: "XENOGENEIC CELL THERAPY CURRENT PROGRESS AND FUTURE DEVELOPMENTS INPORCINE CELL TRANSPLANTATION" CELL TRANSPLANTATION, vol. 7, no. 6, 1998, pages 525-539, XP000891203 ISSN: 0963-6897 page 530, left-hand column, last paragraph page 532, right-hand column, last paragraph	1-26
P,X	WO 00 12138 A (DIACRIN INC) 9 March 2000 (2000-03-09) page 13, line 8 - line 13 examples 2,5	1-26
P,X	MCCORMICK A. L. ET AL.: "Cell surface expression of CD154 inhibits alloantibody responses: A mechanism for the prevention of autoimmune responses against activated T cells?" CELLULAR IMMUNOLOGY, vol. 195, no. 2, 1 August 1999 (1999-08-01), pages 157-161, XP002151134 ISSN: 0008-8749 the whole document	1-26

INTERNATIONAL SEARCH REPORT

...on on patent family members

...ial Application No
PCT/GB 00/02652

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9826061 A	18-06-1998	AU 5795798 A	03-07-1998
		BR 9714004 A	02-05-2000
		CN 1246892 A	08-03-2000
		EP 0948614 A	13-10-1999
		NO 992756 A	09-08-1999
WO 0012138 A	09-03-2000	AU 5699599 A	21-03-2000

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
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15. JUN. 2001 *056810

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	13.06.2001
Applicant's or agent's file reference: P38044WO	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/02652	10/07/2000	23/07/1999

International Patent Classification (IPC) or both national classification and IPC
C12N15/12

Applicant
UNIVERSITY OF SHEFFIELD et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☒	Certain document cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 23/11/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Rojo Romeo, E

Formalities officer (incl. extension of time limits)
Zoglauer, H
Telephone No. +49 89 2399 8051

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, pages:

1-18 as originally filed

Claims, No.:

1-24 as received on 13/02/2001 with letter of 13/02/2001

Drawings, sheets:

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

III. Non- stablishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 10,

because:

   ☒ the said international application, or the said claims Nos. 10 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:
see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

☐ all parts.

☒ the parts relating to claims Nos. 1-9, 11-24.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1, 19, 22-24 (NO)
Inventive step (IS)	Claims	1-9, 11-24 (NO)
Industrial applicability (IA)	Claims	21

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Newly filed claim 10 concerns any vector without the essential technical feature that it should bear the genetic information for CD154. Consequently, claim 10 goes beyond the subject-matter of the application as filed and is thus not acceptable. Claim 10 is thus not examined here.

Re Item IV

Lack of unity of invention

The Applicant's attention is drawn to the fact that since the cell of claim 1 does not have the technical feature "transfected with DNA encoding ... the CD 154 ligand", the use of this cell does not lead to the same technical effect as the use of the cell of claim 2. Thus, an objection for lack of unity arises.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Novelty (Art. 33(2) PCT)

Claim 1 is directed to the use of any cell which does not belong to the immune system (see page 4 of the present application). Such cells were already used in tissue engineering. Thus, claim 1 is not novel.

Claim 19 is directed to an organ which comprises at least one cell which does not naturally express the CD154 ligand. This characteristic applies to any known organ. Consequently, claim 19 is not novel.

Concerning claims 22-24, the Applicant's attention is drawn to the fact that the intention of use does not limit the scope of a claim which is directed to a composition. The claim must be interpreted as being directed to a composition per se regardless of its use. Thus, said claims are read as being directed to a composition containing the cell of claim 1. Compositions containing cells which do not express CD154 are known. Consequently, claims 22-24 are not novel.

In summary, claims 1, 19, 22-24 are not novel, and thus, not inventive.

2. Inventive step (Art. 33(3) PCT)
Reference is made to the following documents cited in the International Search Report:

D1: GAINER A. L. ET AL.: 'IMPROVED SURVIVAL OF BIOLISTICALLY TRANSFECTED MOUSE ISLET ALLOGRAFTS EXPRESSING CTLA4-IG OR SOLUBLE FAS LIGAND' TRANSPLANTATION, vol. 66, no. 2, 27 July 1998 (1998-07-27), pages 194-199, XP000877391 ISSN: 0041-1337

D2: GRUSS H.-J. AND DOWER S. K.: 'Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas' BLOOD, vol. 85, no. 12, 15 June 1995 (1995-06-15), pages 3378-3404, XP002094502 ISSN: 0006-4971

D1 can be considered as the closest prior art since it discloses the expression of members of the TNF family in transfected mouse islet cells leading to a protective effect on allograft survival. The problem underlying the present application is the provision of alternative molecules for expression in cells to be transplanted for protection of the transplanted cells. The solution provided by the present application is the expression of CD154.
D2 reviews the TNF ligand superfamily: they are all essential for T-cell costimulation and activation. Since CD154 was known to belong to the TNF ligand family and T-cells were known to play a pivotal role in transplant destruction, the skilled person would have been prompted to use this molecule as an alternative to CTLA4-Ig or FasL. Consequently, the scope of the present claims is not considered to involve inventive activity.

Consequently, claims 1-9, 11-24 lack inventive step.

3. Industrial applicability (Art. 33(4) PCT)
For the assessment of the present claim 21 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the

manufacture of a medicament for a new medical treatment.

Re Item VI

Certain documents cited

D1: WO 00 12138 A (DIACRIN INC) 9 March 2000 (2000-03-09)
international publication date: 09.03.00
international filing date: 31.08.99
priority data: 31.08.98

Re Item VII

Certain defects in the international application

Claims 22-24 refer to the cell of claim 1. Claim 1 is however a use claim, not a product claim.

Re Item VIII

Certain observations on the international application

1. Clarity (Art. 6 PCT)

1.1 Claim 2 concerns "at least the effective part of the CD154 ligand". In the absence of a reference to a precise sequence, this information does not constitute any technical feature unambiguously interpretable by the skilled person.

A claim to the use of a cell transfected with DNA encoding at least the effective part of CD 154 ligand, without further indication which of the many functions of CD 154 ligand are meant and in view of the fact that the TNF-related cytokines to which CD154 ligand belongs have overlapping functions, does not comply with Art. 5 and 6 PCT. This is firstly because the skilled person would be left guessing whether or not a derivative which fulfils only one of the functions typical of this molecule falls under the scope of the claim. Moreover, the requirement of Art. 5 PCT is not fulfilled if the claim, on the basis of the broadest possible meaning of the functional definition contained in it, relates to an invention which, having regard to the examples and the information given in the patent specification, cannot be performed in the whole area claimed by a person skilled in the art, using common general knowledge, without undue burden.

Moreover, concerning the term "homologue", the Applicant's attention is drawn to the

fact that any protein or nucleic acid can be seen as the "homologue" of any other by a certain number or type of modifications, deletions, additions, substitutions, etc. In the absence of the indication of a percentage of identity over the entire coding sequence of CD154, and in the light of the homologies between the members of the TNF-related cytokines (D2), this term is ambiguous.

1.2 Concerning claim 11, the terms "which is adapted for recombinant expression of the CD154 ligand" does not necessarily imply that the vector used indeed carries the nucleotide sequence encoding the CD154 ligand.

1.3 The objection under 1.2 applies to claim 12. Moreover, claim 12 is formulated as result to be achieved (appropriate...which optimise the expression) since the "expression control sequences" are not defined in terms of technical features.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 February 2001 (01.02.2001)

PCT

(10) International Publication Number
WO 01/07605 A1

(51) International Patent Classification[7]: C12N 15/12, C07K 14/705, C12N 5/10, A61K 35/12, 7/00, A61L 15/00

(21) International Application Number: PCT/GB00/02652

(22) International Filing Date: 10 July 2000 (10.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9917180.3 23 July 1999 (23.07.1999) GB

(71) Applicant *(for all designated States except US)*: UNIVERSITY OF SHEFFIELD [GB/GB]; Western Bank, Sheffield S10 2TN (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: HEATH, Andrew, William [GB/GB]; University of Sheffield, Medical School, Sheffield S10 2RX (GB).

(74) Agent: HARRISON GODDARD FOOTE; Tower House, Merrion Way, Leeds LS2 8PA (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(48) Date of publication of this corrected version:
12 July 2001

(15) Information about Correction:
see PCT Gazette No. 28/2001 of 12 July 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/07605 A1

(54) Title: IMMUNOSUPPRESSION BY CELL SURFACE EXPRESSION OF RECOMBINANT CD154

(57) Abstract: The invention relates to cells and/or tissues and/or organs which do not naturally express the cell surface receptor CD40 ligand, CD154, for use in therapeutic and cosmetic tissue engineering and/ r organ transplantation.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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(43) International Publication Date
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(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF SHEFFIELD** [GB/GB]; Western Bank, Sheffield S10 2TN (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **HEATH, Andrew, William** [GB/GB]; University of Sheffield, Medical School, Sheffield S10 2RX (GB).

(74) Agent: **HARRISON GODDARD FOOTE**; Tower House, Merrion Way, Leeds LS2 8PA (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/07605 A1

(54) Title: CD154 LIGAND, AND RECOMBINANT CELLS EXPRESSING IT

(57) Abstract: The invention relates to cells and/or tissues and/or organs which do not naturally express the cell surface receptor CD40 ligand, CD154, for use in therapeutic and cosmetic tissue engineering and/or organ transplantation.

INTERNATIONAL SEARCH REPORT

Interna[redacted] Application No
PC[redacted] 00/02652

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 C12N5/10 A61K35/12 A61K7/00
A61L15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 26061 A (UNIV CALIFORNIA) 18 June 1998 (1998-06-18) the whole document ---	1-26
A	GAINER A. L. ET AL.: "IMPROVED SURVIVAL OF BIOLISTICALLY TRANSFECTED MOUSE ISLET ALLOGRAFTS EXPRESSING CTLA4-IG OR SOLUBLE FAS LIGAND" TRANSPLANTATION, vol. 66, no. 2, 27 July 1998 (1998-07-27), pages 194-199, XP000877391 ISSN: 0041-1337 the whole document --- -/--	1-26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2000	08/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mandl, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GRUSS H.-J. AND DOWER S. K.: "Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas" BLOOD, vol. 85, no. 12, 15 June 1995 (1995-06-15), pages 3378-3404, XP002094502 ISSN: 0006-4971 the whole document	1-26
A	EDGE A. S. B. ET AL.: "XENOGENEIC CELL THERAPY CURRENT PROGRESS AND FUTURE DEVELOPMENTS INPORCINE CELL TRANSPLANTATION" CELL TRANSPLANTATION, vol. 7, no. 6, 1998, pages 525-539, XP000891203 ISSN: 0963-6897 page 530, left-hand column, last paragraph page 532, right-hand column, last paragraph	1-26
P,X	WO 00 12138 A (DIACRIN INC) 9 March 2000 (2000-03-09) page 13, line 8 - line 13 examples 2,5	1-26
P,X	MCCORMICK A. L. ET AL.: "Cell surface expression of CD154 inhibits alloantibody responses: A mechanism for the prevention of autoimmune responses against activated T cells?" CELLULAR IMMUNOLOGY, vol. 195, no. 2, 1 August 1999 (1999-08-01), pages 157-161, XP002151134 ISSN: 0008-8749 the whole document	1-26

INTERNATIONAL SEARCH REPORT

...information on patent family members

Inte... Application No
PC... 00/02652

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9826061 A	18-06-1998	AU 5795798 A	03-07-1998
		BR 9714004 A	02-05-2000
		CN 1246892 A	08-03-2000
		EP 0948614 A	13-10-1999
		NO 992756 A	09-08-1999
WO 0012138 A	09-03-2000	AU 5699599 A	21-03-2000

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 February 2001 (01.02.2001)

PCT

(10) International Publication Number
WO 01/07605 A1

(51) International Patent Classification[7]: C12N 15/12, C07K 14/705, C12N 5/10, A61K 35/12, 7/00, A61L 15/00

(21) International Application Number: PCT/GB00/02652

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(25) Filing Language: English

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(30) Priority Data:
9917180.3 23 July 1999 (23.07.1999) GB

(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF SHEFFIELD** [GB/GB]; Western Bank, Sheffield S10 2TN (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **HEATH, Andrew, William** [GB/GB]; University of Sheffield, Medical School, Sheffield S10 2RX (GB).

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/07605 A1

(54) Title: CD154 LIGAND, AND RECOMBINANT CELLS EXPRESSING IT

(57) Abstract: The invention relates to cells and/or tissues and/or organs which do not naturally express the cell surface receptor CD40 ligand, CD154, for use in therapeutic and cosmetic tissue engineering and/or organ transplantation.

ATENT COOPERATION TRE Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 27 March 2001 (27.03.01)	
International application No. PCT/GB00/02652	**Applicant's or agent's file reference** P38044WO
International filing date (day/month/year) 10 July 2000 (10.07.00)	**Priority date** (day/month/year) 23 July 1999 (23.07.99)
Applicant HEATH, Andrew, William	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

16 February 2001 (16.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Col mbettes** **1211 Geneva 20, Switz rland**	Authorized officer Zakaria EL KHODARY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PCT ORLD INTELLECTUAL PROPERTY ORGANIZA
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 48/00, A01K 67/027 // C07K 14/705, 14/715, C12N 15/62, 5/10

A3

(11) International Publication Number: WO 00/12138

(43) International Publication Date: 9 March 2000 (09.03.00)

(21) International Application Number: PCT/US99/19915

(22) International Filing Date: 31 August 1999 (31.08.99)

(30) Priority Data:
09/144,006 31 August 1998 (31.08.98) US

(71) Applicant: DIACRIN, INC. [US/US]; Building 96, 13th Street, Charlestown Navy Yard, Charlestown, MA 02129 (US).

(72) Inventor: EDGE, Albert; 4A Kirkland Place, Cambridge, MA 02138 (US).

(74) Agents: MANDRAGOURAS, Amy, E. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(88) Date of publication of the international search report:
22 June 2000 (22.06.00)

(54) Title: CELLS EXPRESSING IMMUNOREGULATORY MOLECULES AND USES THEREFOR

(57) Abstract

Compositions comprising genetically modified cells which express at least one immunoregulatory molecule and methods for using the genetically modified cells are described. The immunoregulatory molecule expressed by the cell(s) are capable of inhibiting T cell activation and/or natural killer cell–mediated immune response against the cell upon transplantation into a recipient subject. The cells of the invention can express an immunoregulatory molecule on the surface of the cells or secrete the immunoregulatory molecule in soluble form. The cells of the invention can be transplanted into a recipient subject such that immune rejection of the cell is inhibited. In addition, non–human transgenic animals which contain cells which are genetically modified to express at least one immunoregulatory molecule are described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

[redacted]ional Application No
[redacted]T/US 99/19915

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K48/00 A01K67/027 //C07K14/705,C07K14/715,C12N15/62, C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 06241 A (GEN HOSPITAL CORP) 20 February 1997 (1997-02-20) page 1, line 24-33 page 6, line 24-29 claims	1,14-18, 21,34-40
A		2,4-13, 19, 23-33,41

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2000	22/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GAINER A L ET AL: "Improved survival of biolistically transfected mouse islet allografts expressing CTLA4-Ig or soluble Fas ligand." TRANSPLANTATION, (1998 JUL 27) 66 (2) 194-9. , XP000877391 abstract page 198, left-hand column, line 18-22 table 2	1,3,20, 22
A	---	2,4-13, 19, 23-33,41
X	SEEBACH JORG D ET AL: "HLA class I expression on porcine endothelial cells protects against human NK cytotoxicity." IVTH INTERNATIONAL WORKSHOP OF THE SOCIETY FOR NATURAL IMMUNITY;HELSINKI, FINLAND; MAY 28-31, 1997, vol. 15, no. 4, 1996, page 176 XP000877395 Natural Immunity 1996-1997 ISSN: 1018-8916 the whole document	1,14-18, 21,34-39
X	--- GRAEB CHRISTIAN ET AL: "Immunologic suppression mediated by genetically modified hepatocytes expressing secreted allo-MHC class I molecules." HUMAN IMMUNOLOGY JULY, 1998, vol. 59, no. 7, July 1998 (1998-07), pages 415-425, XP000877401 ISSN: 0198-8859 abstract page 416, right-hand column, paragraph 2 page 422, right-hand column, paragraph 3 -page 423, left-hand column, paragraph 1	1,14-18, 21,34-39

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/19915

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 20-39 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

tion on patent family members

[redacted] ional Application No
PCT/US 99/19915

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9706241 A	20-02-1997	AU 6687696 A	05-03-1997
		EP 0842266 A	20-05-1998
		JP 11510698 T	21-09-1999

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P38044WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/ 02652	10/07/2000	23/07/1999
Applicant		
UNIVERSITY OF SHEFFIELD		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   IMMUNOSUPPRESSION BY CELL SURFACE EXPRESSION OF RECOMBINANT CD154

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
Harrison Goddard Foote
Tower House
Merrion Way
Leeds LS2 8PA
UNITED KINGDOM

Applicant's or agent's file reference P38044WO	Date of mailing *(day/month/year)* 27/10/2000 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/GB 00/ 02652	International filing date *(day/month/year)* 10/07/2000
Applicant UNIVERSITY OF SHEFFIELD	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Andria Overbeeke-Siepkes

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continu[redacted]

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.